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HORMONAL AND CHOLINERGIC INFLUENCES ON
ACUTE PANCREATITIS IN THE RAT



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HORMONAL AND CHOLINERGIC INFLUENCES ON ACUTE
PANCREATITIS IN THE RAT

by
Anders Evander

Lund 1982

To my family



The present thesis is based on the following papers:

- I. A Evander, E Hederström, B Hultberg and I Ihse.
Exocrine pancreatic secretion in acute experimental pancreatitis.
Accepted for publication in Digestion.
- II. A Evander, I Ihse and I Lundquist.
Influence of hormonal stimulation by caerulein on acute experimental pancreatitis in the rat.
Eur Surg Res 13:257-268, 1981.
- III. A Evander, I Lundquist and I Ihse.
Influence of gastrointestinal hormones on the course of acute experimental pancreatitis.
Accepted for publication in Hepato-Gastroenterology.
- IV. A Evander, I Lundquist and I Ihse.
Influence of intraluminal trypsin activity on the course of acute experimental pancreatitis.
Submitted.
- V. A Evander and I Ihse.
Cimetidine treatment in acute experimental pancreatitis.
Eur Surg Res 12:301-309, 1980.
- VI. A Evander, I Ihse and I Lundquist.
Influence of anticholinergic treatment on rat pancreatic protein and amylase concentrations.
Scand J Gastroent 14:115-117, 1979.
- VII. A Evander, I Lundquist and I Ihse.
Hormonal and cholinergic effects on amylase and lysosomal enzyme activities in pancreatic tissue and ascites of rats with acute experimental pancreatitis.
Submitted.
- VIII. A Evander, I Ihse and I Lundquist.
Hormonal and cholinergic influences on pancreatic lysosomal and digestive enzymes in rats.
Submitted.

The above articles will be referred to in the text by their respective Roman numerals.

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Chapter 1

PURPOSE AND PLAN OF THE PRESENT INVESTIGATION

In an attempt to suppress the inflammatory process in acute pancreatitis different procedures aiming at "rest of the gland" have been used for many years. Various methods to counteract the hormonal and neural stimulation of the gland have been suggested. The main interest has been concentrated on inhibition of pancreatic secretion, whereas the concept of inhibiting pancreatic protein synthesis has been treated somewhat grudgingly, although proteolysis and autolysis are considered to have a central role in the pathophysiology of the disease.

A negative feed-back regulation of pancreatic secretion exerted by intraluminal trypsin seems to exist in the rat (48, 65, 90), pig (66) and probably man (64). Most data suggest that this regulatory mechanism is mediated by gastrointestinal hormones or factors from the intestinal mucosa. Thus, lack of trypsin within the intestinal lumen induces release of gastrointestinal hormones or factors stimulating pancreatic secretion as well as enzyme synthesis. Altered intestinal enzyme milieu caused by changes in pancreatic secretion - due to the pancreatitis *per se* or to different therapeutic measures - may therefore be supposed to influence the course of acute pancreatitis.

The main purpose of the present thesis was to investigate the role of direct or indirect hormonal and neural stimulation on the course of acute experimental pancreatitis with special reference to certain pathogenetic and pathophysiological mechanisms.

1. As the intestinal enzyme milieu may influence acute pancreatitis and as reports on pancreatic secretion in acute pancreatitis are controversial, there was a need to investigate pancreatic secretion in the animal model of acute pancreatitis selected in the present thesis.
2. Although treatment of acute pancreatitis is mainly based on the concept "rest of the gland" little is known about hormonal and neural stimulation of the gland in acute pancreatitis. The next step was therefore to study the effect of direct or indirect hormonal and cholinergic stimulation on the course of the disease.
3. If stimulation of the gland during pancreatitis was found to aggravate the disease it would be of interest to study the effect of inhibitory agents such as anticholinergic or histamine- H_2 -receptor blocking drugs on the outcome of the disease.
4. As the intestinal enzyme (trypsin) milieu is known to influence stimulation of the pancreas in normal rats by inter-

fering with release of gastrointestinal hormones or factors, the effect of variation of the intraluminal trypsin activity on the outcome of the disease was studied.

5. If hormonal or cholinergic stimulation of the gland influences the course of acute pancreatitis it would be of interest to try to elucidate the influence of such stimuli on certain possible pathogenetic and pathophysiological mechanisms. Therefore the influence of hormonal and cholinergic stimulation on lysosomal enzyme activities was studied in pancreatic and normal rats.

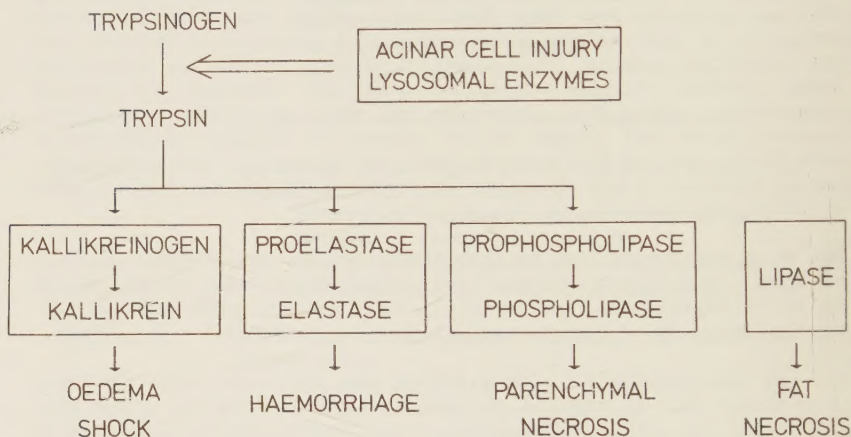


Fig 1. Synopsis of pathogenesis and pathophysiology in acute pancreatitis.

Chapter 2

BACKGROUND

A. Current concepts on the pathogenesis of acute pancreatitis

Acute pancreatitis is recognized as mainly an autodigestive disease (18, 29). Its characteristics are proteolytic destruction of pancreatic substance, necrosis of blood vessels with subsequent haemorrhage, necrosis of fat and an inflammatory reaction (13).

Activation of trypsinogen to trypsin within pancreatic tissue is thought to be an important initiating factor in the disease. The complex pathophysiology of the disease can, however, not be explained by direct tryptic effects only. It also reflects indirect tryptic effects such as activation of the zymogens phospholipase A, proelastase and kallikreinogen (Fig 1).

Phospholipase A hydrolyzes lecithin to lysolecithin, a substance with marked cytotoxic properties. Lysolecithin causes parenchymal coagulation necrosis and liberation of toxic substances from the pancreas to the circulation (106). Therefore, phospholipase A has been suggested as a key enzyme in the pathogenesis of acute pancreatitis (8, 18, 106). Elastase also seems to be of pathophysiological importance by causing destruction of the elastin of the blood vessels causing haemorrhage. Kallikrein induces release of bradykinin, resulting in dilatation of blood vessels, oedema, aggregation of leukocytes and pain (150).

Amylase, nuclease and lipase are secreted from the gland as active enzymes. Amylase and nuclease have no known pathophysiological role in acute pancreatitis, whereas lipase probably is involved in the formation of fat necrosis by hydrolyzing triglycerides to glycerol and free fatty acids, the latter of which combine with calcium to form insoluble soaps. It has, however, also been suggested that fat necroses are caused by activation of intracellular, hormone-sensitive lipase, an enzyme localized in the fat cell (52).

Within the intestine, the activation of trypsinogen to trypsin is mainly caused by the action of enterokinase. Activation of small amounts of trypsinogen probably occurs normally also within pancreatic tissue. Further activation of trypsinogen and other proenzymes is, however, prevented by the existence of different protective mechanisms. Thus, as mentioned above, several of the enzymes are inactive within the pancreas; they are localized and encapsulated in the zymogen granulae and secreted by exocytosis directly into the duct system (100, 161). Also, the epithelium of the ducts is covered by a protective layer of mucopolysaccharides (80, 123), and finally there are protease inhibitors in the pancreatic duct system as well as in the pancreatic parenchyma and in the circulation. These inhibitors combine with the activated enzy-

mes by formation of complexes (14, 21, 107).

The initiating role of trypsinogen activation in the development of acute pancreatitis was long questioned, mainly because of lack of evidence of active trypsin within the pancreatic tissue during the disease. Recently, however, several authors have been able to demonstrate trypsin inhibitor complexes in blood and ascites in patients or experimental animals with pancreatitis (14, 21, 107). Most data therefore speak in favour of the opinion that intrapancreatic activation of trypsin may be an important initiating factor in acute pancreatitis. The cause of activation is not known. However, it has been suggested that injury of the acinar cells and activation of lysosomal enzymes may be involved (Fig 1). It has e.g. been shown that cathepsin D at low pH can cause activation of trypsinogen (49).

Several etiological factors are described in acute pancreatitis. In 1901, Opie (109) presented his now classical theory according to which a stone in the ampulla of Vater permits bile to reflux into the pancreatic duct via a common channel. This theory has, however, been questioned as many patients with bile-pancreatitis at operation or at autopsy are free from stones in the common bile duct. Further, Kelly (76) found that in about 20 % of patients with bile-pancreatitis, who passed gallstones in the faeces within eight days after the onset of pancreatitis, a common channel was not found. Acute pancreatitis has also been suggested to be a result of obstruction at the level of the papilla together with a concomitant stimulation of the gland (91). On the other hand, McCutcheon (98) has suggested that reflux of duodenal juice containing enterokinase into the pancreatic duct seems most logical as an explanation of enzyme activation within the gland.

Alcohol is the most common cause of acute pancreatitis in Sweden (147). The mechanism by which alcohol induces pancreatitis is not known, but it has been suggested that it may act directly on the acinar cell by changing the cell metabolism or that it may stimulate secretion at the same time as it causes oedema and obstruction of the papilla (131). It is an old clinical observation that the disease often starts after a heavy meal with or without alcohol ingestion (112). The idea that overstimulation of the gland has got a pathogenetic importance (91, 112), is also supported by experimental studies (2, 12, 81, 86, 154). Disturbance of the acinar cell metabolism may also be a consequence of the use of different drugs (94), chemical substances (23) or malnutrition (121).

Whether etiological factors such as hyperparathyreoidism, hyperlipidemia or infectious agents (10, 43) cause disturbances of the cell metabolism - or involve other mechanisms - is not known. Also ischemia of the gland (157) and activation of the complement system have been discussed in this respect (138, 139).

In summary, a unified concept for the pathogenesis of acute pancreatitis seems to implicate an initiating role of the activation of trypsinogen within pancreatic parenchyma. This starts a chain of events including activation of other proenzymes such as phospholipase A, proelastase and kallikreinogen, all of which exert effects which can be directly related to clinical manifestations of the disease such as necrosis, haemorrhage, shock and pain. The reason for trypsinogen activation is not known but seems to be associated with injury and/or metabolic disturbances of the acinar cell. In different ways the known etiological factors such as alcohol and cholelithiasis may directly or indirectly have a noxious effect on the acinar cell. Pancreatitis seems not only to be caused by acinary cell injury but also perpetuated by the escape of proteases from damaged acinar cells and thus the process may continue as long as there is a prerequisite for protein synthesis within the gland.

B. Aspects on the management of acute pancreatitis

General principles for therapy of acute pancreatitis have changed markedly ever since the beginning of the century. Initially surgical treatment, was the main therapeutic procedure (103). One reason, of course, was the difficulty to make a diagnosis without laparotomy. The results of the treatment were dismal. The advent of the method for estimation of amylase in urine and blood (164) was a great step forward by increasing the possibilities to make the diagnosis without laparotomy and contributed to a more conservative attitude also of the treatment. The main therapeutic principle became "to put the gland at rest". The introduction of intensive care units resulted in improvement of the management of the patients, especially with regard to fluid and electrolyte therapy. After the report of Watts in 1963 (160) on the treatment with total pancreatectomy of a patient with acute pancreatitis, a renewed interest for surgical treatment arose. Today, the therapeutic approach to acute pancreatitis may be described as a compromise between conservative and surgical treatment, especially after the introduction of peritoneal lavage (47, 155).

There is no therapy that has been proved by controlled clinical trials to alter favourably the course of acute pancreatitis. Clinical trials are, however, difficult both to design, perform and evaluate due to a lack of adequate methods to determine the degree of severity of the disease. With the use of sophisticated statistical analyses several authors have recently put together different prognostic signs which may be of value in this respect (99, 111, 116). Determination of methaemalbumin has also been shown to have some prognostic value (85). Another difficulty in the evaluation of therapeutic methods is that the pattern of etiological factors differs markedly especially when comparing studies from different countries.

As mentioned above, the management of patients with acute pancreatitis today is mainly conservative. General principles of the treatment are to "rest the gland", to replace fluid, electrolyte, protein and blood losses and to institute meticulous monitoring for local and systemic complications. "Rest of the gland" is aimed at by refraining from oral feeding, by nasogastric suction and by administration of different drugs which are thought to inhibit the hormonal and neural stimulation of the gland. For this purpose antacids, anticholinergics and lately the histamine- H_2 -receptor antagonist cimetidine have been attempted. Antacids neutralize the hydrochloric acid within the stomach, whereas cimetidine inhibits its release from the gastric mucosa. Theoretically the increase of duodenal pH may inhibit the release of secretin from the intestinal wall leading to a decreased stimulation of pancreatic secretion. Anticholinergic drugs are further known to inhibit the vagal release of gastrin and to interfere with the vagal innervation of the acinar cell. Due to their ability to inhibit pancreatic secretion calcitonin (135), glucagon (39, 102) and somatostatin (34, 35) have been suggested for the treatment of pancreatitis. Results from both experimental and clinical studies, however, have hitherto not shown any convincing evidence that they have beneficial therapeutic effects (cf Chapter V).

Generally the "rest of the pancreas" has been aimed at by attempts to inhibit pancreatic secretion in different ways. If the disease is in fact caused or maintained by the escape of proteases from damaged acinar cells, it seems illogical to focus only on pancreatic secretion. Inhibition of the synthesis of protein (enzymes) within the gland seems more urgent. Hitherto this aspect has seldom been considered when discussing therapy in acute pancreatitis.

Different enzyme inhibitors have been tried in the treatment of acute pancreatitis. The protease inhibitor Trasylol^R was initially reported to be of value (152) but these promising results were not reproducible in controlled studies (69, 104). As an explanation for the negative results it^R has been proposed that the optimal doses and regimen of Trasylol^R treatment have not yet been defined (70). In experimental studies treatment with the phospholipase inhibitor xylocain (lidocaine chloride) (136) have had some effect in acute pancreatitis. Reports on clinical studies are still lacking.

Drainage of the thoracic duct as a way to clear enzymes from the circulation has been suggested and tested experimentally in acute pancreatitis (134). Once again, documentation from controlled clinical studies is missing.

The fat necroses are considered partly to explain the severe pain in acute pancreatitis. Hallberg and Theve (52) suggested insulin and glucose infusion as a treatment with the idea of inhibiting lipolysis by preventing the activation of the hormone-sensitive lipase in the fat cells. In a small controlled clinical study,

Svensson (147) found some evidence for pain relieving effects of insulin treatment in the disease. In patients with severe pain, epidural anaesthesia seems to be a good alternative. On the whole it seems important to give sufficient analgesics for pain, and potent drugs of the morphine-type are generally needed.

The marked extravasation of fluid, plasma and electrolytes in acute pancreatitis causes - like the release of toxic substances from the gland - a state of shock in untreated cases. It is therefore recommended that patients with severe acute pancreatitis should be managed in intensive care units (30). The use of central venous catheters and Swan-Ganz pressure monitors is important as is the assessment of urinary output and electrolyte and metabolic changes. In case of renal insufficiency peritoneal - or even hemodialysis - may be required. It is also necessary to register arterial oxygen tension repeatedly. Ventilator treatment should be used early in case of deteriorating respiration (68). Attention should be paid on the risk of disseminated intravascular coagulation. Insulin treatment often is needed as is treatment with calcium gluconate. Intensive care is perhaps the single, most important factor which has reduced the mortality.

In 1965 Wall reported dramatic improvement in three patients who underwent peritoneal dialysis for renal insufficiency in acute haemorrhagic pancreatitis (155). Since then several uncontrolled studies have been published stressing the value of this treatment (14, 47). Theoretically, peritoneal lavage is an attractive treatment in acute pancreatitis. Hitherto, however, no adequate prospective controlled clinical studies are published (117, 142). In an ongoing, randomized, prospective controlled study, hitherto comprising 43 patients, the interim results do not unequivocally support previous impressions of beneficial effects of peritoneal lavage (67).

Today it is usually possible to control the initial - often hypovolemic - shock in patients with acute severe pancreatitis. The septic complications are nowadays regarded as the main hazard, occurring 2-3 weeks after the onset of the disease. The value of antibiotic therapy in acute pancreatitis is controversial. No studies have hitherto proved any value of prophylactic chemotherapy (42, 60). In patients with sepsis chemotherapy, however, is necessary and should be instituted according to results of bacterial cultures and sensitivity tests.

Surgical intervention is necessary when the diagnosis is not otherwise verified. Also in patients who suffer from late complications such as intraabdominal abscesses, laparotomy should be done, mainly for drainage (120). A preoperative localization of the abscess or abscesses by means of e.g. computed tomographic scanning is often of value (71). In case of pseudocyst formation, internal drainage is recommended after 6-8 weeks when the cyst wall is mature (28).

Recently, with the use of ultrasound, it has been found that about 20 % of pancreatic pseudocysts undergo a spontaneous resolution (129) making surgery unnecessary.

In gall-stone pancreatitis it is still controversial whether the gall-stones should be removed during the acute attack. Generally it has been recommended that the operation should be postponed until the attack has subsided. Recently, however, Acosta et al (1) and Stone et al (143) advocated cholecystectomy or even choledocholithotomy within 73 hours after the admission. On the other hand, Ranson (119), Kelly (77) and Osborne et al (111) suggest that the optimal time of surgery is when the acute phase of the disease has subsided, but the patient should not leave the hospital before having an operation. Safrany and Cotton (128) have carried out endoscopic sphincterotomy during the acute attack. The experiences of this new approach are, however, still limited.

Another controversy in the treatment of acute pancreatitis is whether acute resection is justifiable. Although subtotal as well as total pancreatectomy have some advocates (5, 79, 160) the general opinion today seems to favour the avoidance of these heroic procedures mainly because of high morbidity and mortality.

In summary, the treatment of acute pancreatitis is primarily concerned with a meticulous monitoring of fluid and electrolyte balance, respiratory, renal and cardiac function and careful observation of metabolic and hematological disturbances. Pain relief is of utmost importance. Gastric intubation is thought to be of value only in severe cases with signs of paralytic ileus. Antibiotic treatment is recommended only when the patient has developed septic complications and has not been found to be of value as prophylaxis. Surgical intervention is used when the diagnosis is questioned and in cases of late complications such as abdominal abscesses or pseudocysts.

Different procedures have been suggested with the aim of inhibiting or depressing the inflammatory process *per se*. Hitherto, there is no such treatment which has been shown to be of any significant clinical value. Perhaps this may be associated with the fact that the practical application of the concept of rest of the pancreas has been focused mainly on inhibition of pancreatic secretion. On the other hand, inhibition of protein synthesis within the pancreas has attracted little interest, especially when taking into account that autodigestion and proteolysis are important features of the pathophysiology of acute pancreatitis.

C. Animal models for studies of acute pancreatitis

General aspects

Experimentally induced acute pancreatitis was first described

comprehensible by Claude Bernard in 1856 (20). He infused olive oil into the pancreatic duct of dogs and observed a high mortality rate. At autopsy a marked peritonitis was found with diffuse necroses of the pancreatic gland and with fat necrosis in the abdominal cavity. After Opie had presented his theory of the possible association between gallstones and acute pancreatitis injection of bile into the pancreatic duct was used as a way to induce experimental pancreatitis. Different other methods for induction of acute experimental pancreatitis have been attempted, mainly based on new approaches to the pathogenesis of the disease (7, 98, 127).

In experiments with acute pancreatitis cat, dog, guinea-pig, pig, mouse, rabbit and rat have generally been used. In several respects there are differences between these animal models and therefore it is of importance to have species differences in mind as well as the method used for induction of the disease when comparing results from different studies.

The different techniques of inducing experimental pancreatitis are intraductal or interstitial injection of different drugs and agents, interference with the circulation of the gland and measures causing metabolic disturbances. For intraductal injection bile or bile-salt solutions (84), elastase (46), trypsin or chymotrypsin (156), lipase, phospholipase, lactic acid, olive or oleic oil (84) and bacterial toxins (149) have been used. Sometimes the injection has been combined with ligation of the bile-pancreatic duct and/or hormonal stimulation of the pancreatic secretion. Pfeffer has suggested a "closed duodenal loop" as a suitable model for induction of pancreatitis. The idea is that the pancreatitis is caused by duodenal reflux into the pancreatic duct (26, 113).

In guinea-pig interstitial injection of bile-salts and trypsin (110) has been used for induction of pancreatitis. Using this model, however, it is especially difficult to standardize the technique and further the pancreatitis induced has got few similarities with the human disease. When induction of pancreatitis by ischemia has been used, embolization of the arteries to the pancreas has been performed, e.g. with microspheres. Also induction of thrombosis of the vessels has been suggested (55, 114). If methionine and choline are excluded from the diet and ethionine added, metabolic changes will occur in the pancreas, changing the permeability of the membranes of the acinar cells, leading to enzyme leakage and enzyme activation. This method has generally been used in mice (121) but also sometimes in rats (97).

Even with a strict standardization of the experimental design the degree of severity of the disease varies fairly much and it is generally difficult in repeated experiments to achieve the same severity of the disease. Therefore it is of utmost importance to use control groups in every study (84, 144, 163). Parameters generally used are survival rate, changes in S-amylase, histopatholo-

gical changes, occurrence and distribution of fat necrosis, estimation of digestive enzymes within the pancreatic tissue, the occurrence, volume and enzyme activities of peritoneal fluid and finally different laboratory tests such as haemoglobin, haematocrit, calcium, creatinine, leukocytes etc. Protease-inhibitor complexes within peritoneal fluid, serum and lymph have also been used (107).

Few authors have reported more than 2-3 parameters in a single experiment. Survival seems to be the only parameter of reasonable prognostic value in experimental series hitherto presented. The degree of standardization of the models is, however, generally not possible to deduce from the articles. Therefore, there is a need for evaluation of survival rate as well as other parameters in studies where a meticulous standardization has been used.

Experimental rat models

The rat is one of the most common species used for studies on experimental pancreatitis. The reasons for this are that the animal is easy to handle, that it is easy to get inbred groups of animals, that the normal physiology of the rat is extensively studied, and that the rat also for economical reasons, well may be used.

The pancreas of the rat is fan-shaped, has a scattered localization within the duodenal mesentery, and extends towards the liver and the spleen. About 12-15 pancreatic ducts are draining separately into the combined bile-pancreatic duct. The rat has no gallbladder.

The most common way to induce pancreatitis in the rat is by injection or infusion of bile or bile-salt with or without trypsin into the common bile-pancreatic duct. Sometimes olive oil is used.

The parameters generally used in the rat when studying acute pancreatitis are the same as those mentioned above for other species. When - as in the present thesis - using injection into the bile-pancreatic duct of sodium taurodeoxycholate (NaTDC) and trypsin, Aho et al (3, 4) found initial signs of oedema and bleedings close to the duct system. After about one minute there were signs of dissolution of the bile ducts and desorganization of vessels with formation of thromboses, poikilocytosis and extravasal leakage of red blood cells. The nuclei of the acinar cells became pyknotic, and their cytoplasm lost its basophilic staining. Neutrophils and eosinophils were seen in the interstitium. After about one hour peritoneal fluid started to appear. Fat necroses were seen after six hours. It was concluded that the initial cell injury was caused by the detergent effect of bile-salts. By using intravital microscopy Storck (144) found changes in the fat cell and appearance of fat necroses already 35-50 minutes after induction of pancreatitis using intraductal infusion of sodium taurodeoxycholate (NaTC).

Chapter 3

MATERIAL AND METHODS

Animals

Male Wistar rats weighing 250-300 g were used. The animals had free access to tap water and were kept on a standard pellet diet, which was withdrawn 12-24 hours before the start of the experiments. The animals were housed five per cage. Lights were on between 7.00 a.m. and 7.00 p.m. daily.

Chemicals

Sodium taurodeoxycholate (NaTDC) synthesized according to Hofmann (59) was kindly provided by Professor Bengt Borgström, Lund. Crystalline trypsin (Trypure^R) was obtained from Novo A/S, Bagsvaerd, Denmark. Caerulein, a synthetic decapeptide, structurally closely related to cholecystokinin-pancreozymin (CCK-PZ) was put at our disposal by Pharmitalia Research Institute, Milano, Italy. This drug has a potent stimulatory effect on the exocrine pancreatic secretion (45). Secretin from hog prepared according to Jorpes and Mutt (74) was obtained from AB Kabi, Stockholm, Sweden. Carbachol was obtained from British Drug Houses Ltd., Poole, England. Standard pellets composed according to actual international specifications were delivered by Anticimex AB, Stockholm, Sweden, and contained the following raw materials: fish protein concentrate, soy meal (roasted), fodder yeast, cereals, wheat sprouts, animal fat, soy oil, vitamin concentrate and minerals. Olive oil was obtained from the local hospital pharmacy. The trypsin inhibitor Trasylol^R was delivered by Bayer AG, Leverkusen, Western Germany. The histamine-H₂-receptor antagonist cimetidine was obtained from SK&F Laboratories Inc., Welwyn Garden City, England. The anticholinergic drug propantheline bromide (Pro-Banthine^R) was delivered by G.D. Searle & Co., Chicago, Ill., USA. Isopaque 260 mg Io/ml was obtained from Nyegaard & Co. A/S, Oslo, Norway.

Laboratory techniques

Protein concentration in pancreatic homogenates, peritoneal fluid, pancreatic or bile-pancreatic juice was determined as described by Lowry et al (92). Amylase activity in serum, peritoneal fluid and pancreatic or bile-pancreatic juice was determined by the Phadebas test (Pharmacia, Uppsala, Sweden). Amylase activity in pancreatic homogenates was assayed according to the method described by Dahlqvist (31) and lipase by a scaled-down version of the method described by Erlanson and Borgström (40). Trypsin activable trypsin activity (trypsinogen) was measured according to Arnesjö and Grubb (9), using β -toluene sulphonyl L-arginine methyl ester (TAME) as the substrate. Cathepsin D was determined, essentially according to

Barrett (16) as previously described (88). Acid phosphatase and N-acetyl- β -D-glucosaminidase were determined with methyl-umbelliferyl-linked substrates as previously described (88, 89). Radio-immunological determination of insulin in plasma was performed according to Heding (58). Determination of serum glucose was performed enzymatically (glucose oxidase) (96). Bicarbonate in pancreatic or bile-pancreatic juice was assayed indirectly from pH and $p\text{CO}_2$ (140). Gastric acid secretion was determined titrimetrically (62).

Experimental procedures

Induction of pancreatitis

After at least 12 hours' fasting but with free access to tap water until two hours before the operation the rats were operated upon under light ether anesthesia. After a short midline incision, the duodenal loop and bile-pancreatic duct were identified and gently held up. Using a thin polyethylene catheter the bile-pancreatic duct was cannulated transduodenally. The catheter was introduced not more than five mm. A clamp was placed over the duodenal orifice in order to prevent reflux during infusions. Using an infusion pump 0.5 ml 0.025 M glycyl-glycine-NaOH buffer, pH 8.0, containing 0.02 M CaCl_2 and 10 μmol of sodium taurodeoxycholate (NaTDC), and varying amounts of trypsin (1, 2 and 10 nmol) were injected into the ducts. Increasing amounts of trypsin added to the buffer solution were used in order to get a pancreatitis of different severity (Fig 2). The infusion was given during one minute. In order to prevent infusion into the intrahepatic ducts a clamp was placed on the duct as close to the liver as possible during the infusion. After removal of the catheter the skin incision was closed using 4-0 suture-amide.

In some experiments, repeated blood samples were drawn by puncture of the retrobulbar plexus (126) in unanaesthetized animals.

At the end of the experimental period the animals were sacrificed by an overdose of ether. Via a midline incision access to the abdominal cavity was obtained. In case of ascites, its volume was determined and samples taken for assay of protein concentration and enzyme activities.

A blind macroscopic evaluation of the degree of the pancreatitis was performed (i.e. the observer was unaware of the specific treatment given to each rat; 0 = no pathological findings, 1 = oedema of the pancreas, 2 = oedema and fat necrosis, 3 = oedema, fat necrosis and areas of bleeding, 4 = oedema, fat necrosis, areas of bleeding and necrosis of the pancreas). The pancreases were rapidly removed, and in some rats a piece, always from the same anatomical localization, was taken for histological examination. The rest of the pancreas was immediately frozen at -20°C and later lyophilized for

at least 48 hours. Protein concentration and enzyme activities in pancreatic tissue were determined. Further, in some experiments the water content (%) of the pancreas was calculated.

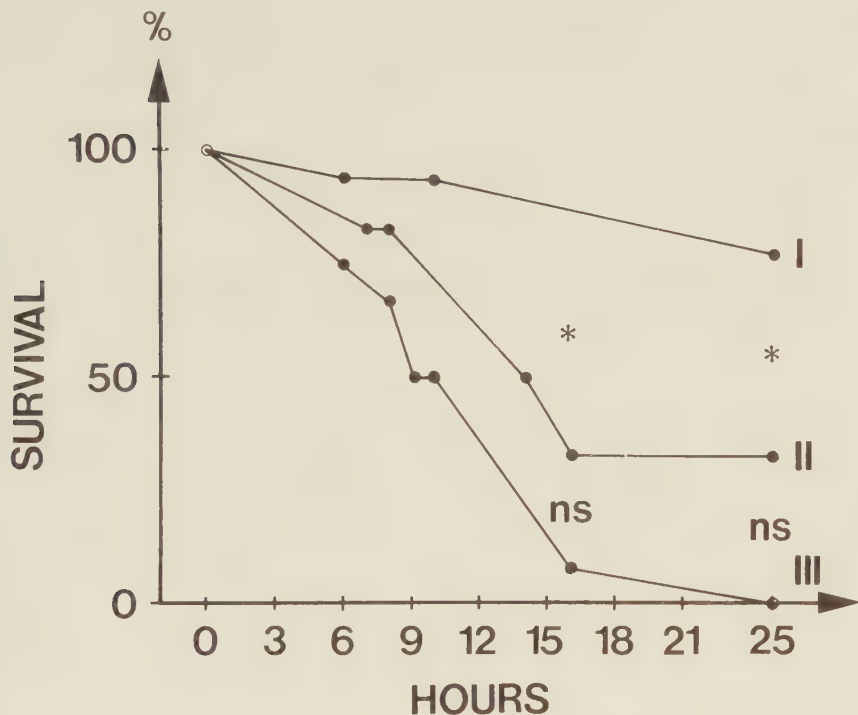


Fig 2. Survival rates in three rat groups with acute experimental pancreatitis induced by injecting buffer solution, NaTDC and varying amounts of trypsin (group I, $n = 18$, 1 nmol; group II, $n = 6$, 2 nmol; group III, $n = 12$, 10 nmol of trypsin) into the bile-pancreatic duct. Probability level of random difference is marked by $x = p < 0.05$. Reprinted from paper II.

Experimental model for studies of pancreatic secretion

In the experiments on pancreatic or bile-pancreatic juice secretion the rats were operated on after an overnight fast. Via a midline incision a polyethylene catheter, PE 60, with an inner diameter of 0.76 mm, was introduced transduodenally five mm into the bile-pancreatic duct. A silk ligature was placed around the bile-pancreatic duct in order to maintain the position of the catheter. In studies on pure pancreatic juice secretion the bile duct was doubly ligated as close to the liver as possible. In all rats except for those described in paper VIII a gastric fistula was established. In some rats a catheter was introduced into the femoral vein. The catheters were placed subcutaneously and taken out through the skin close to the tail. After closure of the abdominal wall incision, the animals were placed in restraining cages. After surgical recovery the experiments were started and pancreatic or bile-pancreatic juice collected in tubes placed on ice. After intravenous or subcutaneous injections the influence of different drugs on pancreatic or bile-pancreatic secretion was studied.

Acute pancreatitis was induced after a period of basal sampling. Via the sampling catheter 0.5 ml buffer solution containing 10 μ mol of NaTDC and 1 nmol of trypsin was infused during one minute using an infusion pump. In the rats, where bile-pancreatic juice secretion was studied, a clamp was placed on the bile duct as close to the liver as possible during the infusion. Therefore these rats were anesthetized by repeated intraperitoneal injections of 5 % choral hydrate and kept on the operation table during the whole experimental period.

In order to rule out dislodgement of the catheter methylene blue was injected via the pancreatic fistula at the end of each experiment to check that blue staining of the entire pancreas was achieved.

Volume, protein concentration, amylase and cathepsin D activities and bicarbonate concentration of the secretion were estimated.

Radiological examination of the pancreatic duct system

In one group of rats pancreatitis was induced by infusion of NaTDC-trypsin buffer solution into the pancreatic ducts. In a second group only the buffer solution was injected, and a third group comprised sham-operated rats. Six hours postoperatively the bile-pancreatic duct was re-cannulated and contrast medium slowly injected. Radiological examinations of the gland were performed after injection of 0.05, 0.10 and 0.20 ml, respectively.

Experimental model for studies of gastric acid secretion

Rats were provided with gastric fistulae as described by Bel et al

(19). After surgical recovery and adaptation to the restraining cages, gastric acid output was collected at hourly intervals. Cimetidine or saline was injected subcutaneously (paper V) and the effect on gastric acid secretion studied.

Statistical methods

The data were evaluated by means of Student's t-test, and in the case of survival rates by Fischer's exact test.

PANCREATIC SECRETION IN ACUTE PANCREATITIS*

In the treatment of acute pancreatitis, "rest of the pancreas" has been aimed at by using methods which primarily cause an inhibition of pancreatic secretion. It is, however, noteworthy that our knowledge of pancreatic secretion during pancreatitis is rather scanty and controversial (54, 93, 95, 124, 125, 153). Based on the concept of feed-back regulation of pancreatic secretion exerted by intraluminal trypsin (48, 64, 65, 66), it may be assumed that decrease or abolishment of pancreatic secretion into the intestine may have aggravating effects on the disease caused by an increased and sustained release of gastrointestinal hormones or factors.

Using the experimental model described in Chapter 3, secretion of pure pancreatic juice was found to be stable during the seven hour long experimental period in healthy rats. As shown in Fig 3, induction of pancreatitis by injection of a solution containing NaTDC and trypsin into the pancreatic duct system caused a marked - but not complete - inhibition of pancreatic secretory volume as well as protein output. Exogenous stimulation with caerulein or secretin or a combination of the two hormones did not influence the secretion. Also when studying the combined bile-pancreatic juice, the secretion was stable throughout the experimental period in control rats. Induction of pancreatitis caused a rapid and significant reduction of secretory volume and a gradual decrease of protein output. Also amylase output was rapidly and markedly reduced whereas bicarbonate output was unaffected.

Hansson et al (54) using a similar method as the one used in the present investigation also studied pancreatic secretion in acute pancreatitis. They found that volume as well as protein concentration decreased markedly after induction of pancreatitis and found further that in surviving rats there was a gradual restitution of the secretion. In the dog, Turner et al (153) found a marked reduction in pancreatic secretion during five hours after induction of the disease.

Manabe and Steer (95) studied pancreatic enzyme secretion in acute pancreatitis in an in vitro system in mice. In this study pancreatitis was induced by feeding mice a choline-deficient, ethionine-rich diet for 24 hours. They found an increased output into the medium of protein, trypsinogen, chymotrypsinogen and amylase, and noted a concomitant increase of these enzymes in the pancreatic tissue in comparison with normal mice. Contrary to Manabe and Steer

* The results presented refer to those of paper I.

PANCREATIC JUICE

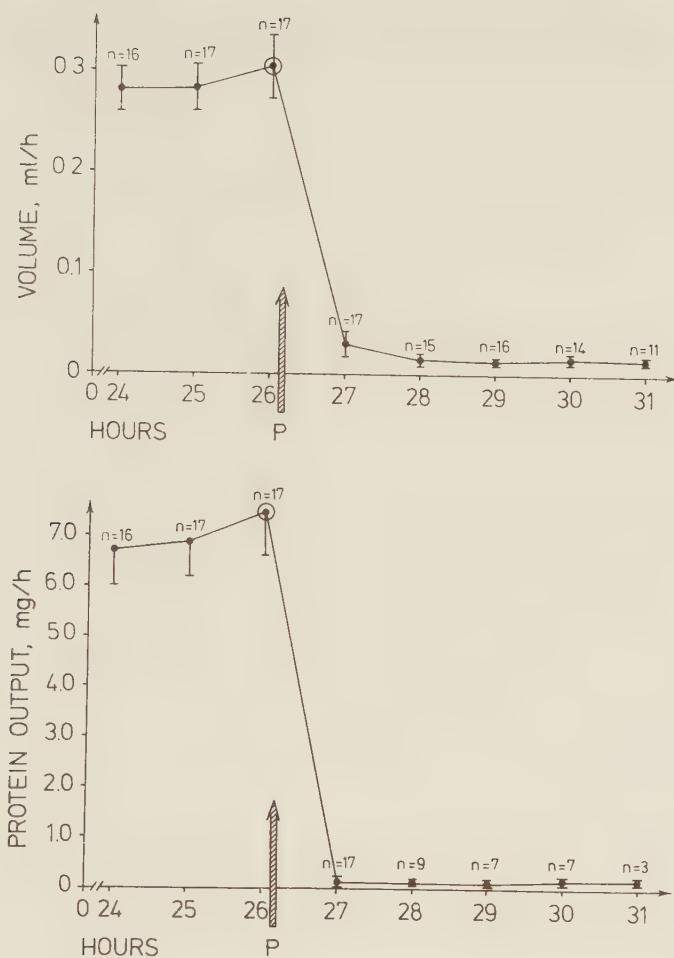


Fig 3. Secretory volume (ml/h) and protein output (mg/h) in rats before and after induction of pancreatitis (P). The number of samples at each point is indicated in the figure. Mean $\pm$ SEM. Reprinted from paper I.

Kimura et al (78) when studying pancreatic secretion in an isolated, ex-vivo-perfused dog pancreas found a gradually decreasing pancreatic secretory volume during four hours after the induction of pancreatitis by intraarterial administration of oleic acid.

Thus, the results from the few experimental studies done on pancreatic secretion in acute pancreatitis are contradictory. It seems as if the model used in the present thesis and the model used by Hansson et al (54) compare best with the conditions in human pancreatitis. Theoretically, one factor contributing to the decreased secretion in pancreatitis may be mechanical obstruction of the pancreatic duct system. In the present study, however, pancreatographic studies did not support this hypothesis but rather speak in favour of the assumption that the decrease in pancreatic secretion is related to ductal and parenchymal injury. Another theoretical possibility is that the low pancreatic secretion into the intestine is a consequence of interstitial oedema due to ductal obstruction with undiminished or even increased secretory capacity of the acinar cells. Against this idea speak electron microscopic studies (4), showing that the acinar cells in sodium taurocholate-induced pancreatitis reveal early injuries with mitochondrial swelling and dissociation of cellular membranes. Although a concomitant occurrence of normal, partly injured and totally necrotic acinar cells was observed, this does not exclude a marked inhibition of pancreatic secretion in acute pancreatitis. In man, Lundh (93) found low trypsin levels in duodenum in 41 examinations of 31 patients with acute pancreatitis, using the Lundh-Borgström test meal. In this study information is not given about the timing of the test meal investigation related to the start of the disease. Nor is the degree of severity of the pancreatitis reported. Regan et al (124) found, contrary to Lundh, normal or even increased pancreatic secretion in two out of three patients with pancreatitis using duodenal intubation. They performed their study 1 to 2 weeks after the start of the disease, which was of rather moderate degree of severity (personal communication). The divergent results of Lundh (93) and Regan et al (124) are probably due to different time intervals from onset of disease until the tests were done and differences in severity.

With the use of endoscopic retrograde cholangio-pancreaticography (ERCP) a direct collection of pancreatic secretion during pancreatitis is possible. Renner et al (125) found normal secretory volume and increased protein concentration in pancreatic juice in two patients with acute pancreatitis and jaundice.

In summary, it seems most plausible, although available results are contradictory, that the exocrine pancreatic secretion is markedly reduced - but not completely abolished - during acute pancreatitis. Mechanical obstruction seems not to be the major cause of the reduced secretion but rather a decreased secretory capacity by the injured acinar cells. This is of particular interest as decreased

pancreatic secretion to the duodenum - resulting in low duodenal trypsin activities - may be a factor which by causing increased hormonal stimulation of the gland may contribute to the maintenance of proteolysis and autolysis during pancreatitis. This may be another reason why measures inhibiting pancreatic secretion in acute pancreatitis have failed to influence the course of acute pancreatitis favourably. Instead, measures which increase intra-duodenal tryptic activity during pancreatitis would be worthwhile to investigate.

Chapter 5

INFLUENCE OF HORMONAL STIMULATION ON ACUTE PANCREATITIS^{*}

The exocrine pancreas consists of acinar cells and ductal cells, the former being in great majority (95 %). Acinar cells synthesize and secrete proteins (digestive enzymes), whereas the electrolytes and water of pancreatic juice are derived from the ductal cells. It has been shown by radioautography and electron microscopy that the enzymatic proteins are synthesized on the rough endoplasmic reticulum (RER). The proteins are contained within the lumen of the RER, and then transported to the Golgi apparatus, where packaging into zymogen granules occurs. Initiation of secretion by different agents results in a process of zymogen extrusion (exocytosis) at the apical part of the cell.

The gastrointestinal hormones cholecystokinin, gastrin, secretin and vasoactive intestinal polypeptide (VIP) are all known as stimulatory agents of the acinar cells (100, 161). These hormones act by binding to receptors on the cell surface. Different receptors, recognizing different hormones, have been identified. Thus, binding of cholecystokinin (CCK) to the receptor results in release of calcium from an intracellular binding site(s) and to formation of cyclic 3,5-GMP. The CCK-receptor also recognizes and attracts gastrin resulting in similar, but less pronounced, intracellular events. Secretin and VIP have probably a common receptor. Binding to this receptor results in increased formation of cyclic 3,5-AMP. Stimulation with CCK results in both secretion and synthesis of enzymes. Some hormones of gastrointestinal origin, e.g. somatostatin, glucagon and pancreatic polypeptide, have an inhibitory effect on secretory processes of the acinar cell (34, 35, 39, 50, 102). Calcitonin has been reported to inhibit pancreatic secretion (135). Further vasopressin is known to inhibit secretion by reducing the blood flow to the acinar cells (132).

Secretion of electrolytes and water by the ductal cells is stimulated by secretin. Atropine does not inhibit the influence of secretin stimulation on the ductal cells.

Effects of hormonal stimulation on the course of acute pancreatitis

A prominent feature in the treatment of acute pancreatitis has been attempts to inhibit the hormonal stimulation of the gland. This principle is based mainly on the assumption that stimulation of the pancreas is harmful during acute pancreatitis. To my knowledge it has, however, neither been questioned nor studied if this principle is correct.

^{*} The results presented refer to those of papers II, III, IV, VII and VIII.

This investigation includes studies of the influence of exogenous stimulation of the pancreas during acute pancreatitis using the CCK-analogue caerulein and secretin. The importance of endogenous release of gastrointestinal hormones and/or factors has also been investigated.

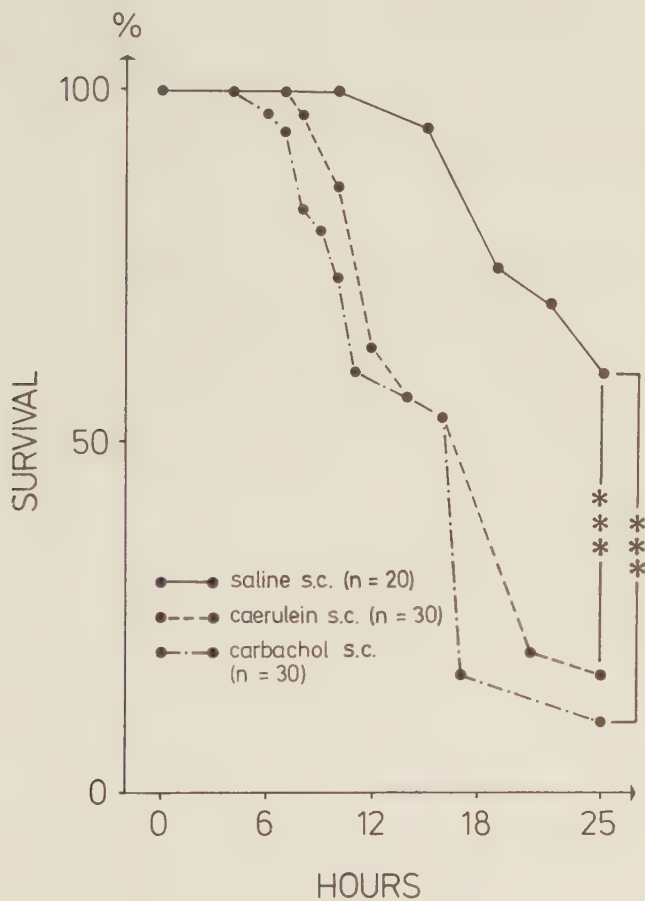


Fig 4. Survival rates in three rat groups with acute experimental pancreatitis given subcutaneous injections of either saline, caerulein or carbachol. Probability level of random difference is marked by xxx = $p < 0.001$. Reprinted from paper VII.

In repeated experiments in rats with moderate pancreatitis administration of caerulein given subcutaneously immediately after induction of pancreatitis and after another 3, 6 and 9 hours (Fig 4) caused a significantly increased mortality rate, whereas secretin administration given in the same way and time intervals did not affect mortality at all. In several experiments oral administration (gastric intubation) of a light pellet meal or of olive oil in order to cause endogenous CCK-release did not influence the mortality rate in rats with pancreatitis. In several experiments oral administration of trypsin inhibitor - a potent stimulator of pancreatic enzyme secretion probably by a release of CCK-like factors - resulted in an increased mortality rate. On the other hand, administration of hydrochloric acid did not affect survival at all in acute pancreatitis.

In all experiments S-amylase (serum amylase) was measured before and after induction of pancreatitis and all animals displayed increased levels of this enzyme during the disease. In these experiments the rise of amylase was of a similar magnitude irrespective of the way of hormonal stimulation used. There was no correlation between S-amylase values and mortality rate, suggesting that S-amylase is of no prognostic value in experimental pancreatitis. In the experiments where caerulein was given to pancreatic rats the activities of amylase and lipase decreased whereas the activities of the lysosomal enzymes cathepsin D and acid phosphatase were increased in pancreatic tissue. Further, in ascites the activities of amylase and cathepsin D were markedly increased. In rats given oral trypsin inhibitor during pancreatitis a tendency to decreased amylase activities within pancreatic tissue and increased activities in ascites was observed. After secretin stimulation the only enzyme change found was an increase of acid phosphatase in pancreatic homogenates. In all experiments a blind macroscopic evaluation was also performed as described above (cf Chapter 3). After caerulein administration the macroscopic finding revealed an aggravation of the disease using the standardized method of evaluation. Otherwise no differences were found in this respect. Apart from mortality rate, the blind macroscopic evaluation and the levels of digestive enzymes and cathepsin D within pancreatic tissue and ascites seemed to give a reflection of the severity of the disease. After oral ingestion of trypsin inhibitor - which is thought partially to act by the same mechanisms as the one exerted by exogenous caerulein (63) - there was a tendency to decreased amylase activities within pancreatic tissue but not in ascites (vide supra). Otherwise rats given trypsin inhibitor did not display similar changes as rats given caerulein regarding the results of macroscopic evaluation. This discrepancy probably reflects differences in the levels of different G-I hormones within the circulation in the two types of experiments.

Although hormonal influences on acute pancreatitis have been sparsely studied previously, different agents with the ability to

inhibit pancreatic secretion have been attempted and suggested as a treatment in pancreatitis. In one study pigs with induced pancreatitis displayed a lower mortality rate after glucagon treatment (158). On the other hand, glucagon administration to dogs (128) or rats (38, 82) with pancreatitis was without beneficial effects. Manabe and Steer (95) induced pancreatitis in mice by administration of an ethionine-supplemented, choline-deficient diet and found that pre-treatment with glucagon caused a decreased mortality rate whereas glucagon administration in connection with the induction was without effects. In an uncontrolled study Stremmel (145) reported improved survival in patients with pancreatitis given glucagon. Subsequent controlled studies (104, 108, 124) have not been able to verify these results. In a Finnish study promising results were reported in 1980 from a double-blind study of the effects of zink-protamine-glucagon versus placebo, but the authors did not find indications for a routine use of glucagon in human acute pancreatitis (75).

In 1976, Dollinger et al (34) suggested somatostatin as a possible useful adjunct in acute pancreatitis. Domschke et al (35) also performed secretory studies in connection with ERCP-examinations and found inhibition of pancreatic secretin-stimulated secretion by somatostatin and suggested that it may be attempted in pancreatitis. Lankisch et al (83) used somatostatin in acute experimental pancreatitis in the rat without finding any evidence for beneficial effects, whereas Schwedes et al (137) after administration of somatostatin found an improvement of the general condition and less pronounced histological changes in dogs with pancreatitis.

The role of calcitonin in acute pancreatitis has been only sparsely studied hitherto, and it is difficult to make any conclusions regarding its effect. In experimental studies vasopressin (6, 132) has been found to exert beneficial effects on survival, histological changes, volume and amylase concentrations of ascites. Controlled human studies are, however, still lacking. Infusion of insulin and glucose have been used by some authors (52) in order to inhibit the formation of fat necrosis. In a small controlled study Svensson (146) reported this treatment to be of some value, but further documentation is necessary.

In most studies on hormonal influences on acute experimental pancreatitis referred to above, mortality is the main parameter studied. Some authors have used microscopical findings and related them to the degree of severity of the disease. It should, however, be noted that the morphology of pancreatitis is dependent on the model for induction of experimental pancreatitis used and that the disease generally is patchy. In ancillary experiments in the present study histological examinations were performed, but it was found difficult to relate the findings to the severity of the disease.

Manabe and Steer (95) studied the activities of amylase, trypsinogen and chymotrypsinogen in pancreatic tissue and found that glucagon treatment caused decreased levels of these enzymes. It must, however, be mentioned that the activities were given per g wet weight. As oedema of the gland is a prominent feature of acute pancreatitis different enzyme activities given per g pancreatic wet weight should be interpreted with caution. Accordingly observed differences of enzyme activities may be due to oedema only. Lankisch et al (83) did not find any influence of somatostatin therapy on the activities of amylase and lipase in ascites nor on the activities of amylase and trypsin in pancreatic tissue. In dogs with induced pancreatitis Shapiro et al (132) found that vasopressin decreased the volume of ascites and its activities of amylase. In the present thesis, in which a highly standardized model for induction and evaluation of pancreatitis was used, macroscopical evaluation was found to compare rather well with mortality rate when this was altered by increasing the amount of trypsin injected together with NaTDC into the bile-pancreatic duct system of rats. Further, if pancreatitis was aggravated by exogenous caerulein administration, there was also a correlation between the mortality rate and the macroscopic evaluation. In this latter group of rats it was found that the activities of cathepsin D in pancreatic tissue and the activities of amylase and cathepsin D in ascites were higher in groups with higher mortality rates.

The present study also comprises studies on the plasma insulin levels during acute pancreatitis, primarily as an attempt to study if they could be of any prognostic value in the disease. A marked increase of the insulin levels was noted 25 hours after induction of pancreatitis. Stimulation with caerulein causing increased mortality rate did not affect the plasma insulin levels as compared to saline-treated pancreatic controls. The results suggest a state of hyperinsulinism in the early stages of acute pancreatitis and are in accordance with the findings by Satake et al (130) and Pi-Sunyer et al (115). It is probable that the increased insulin levels are a consequence of leakage of insulin from the injured beta cells. These findings are also consistent with the findings of increased glucagon levels in plasma found in acute pancreatitis (36, 159).

In summary, the results of the present study suggest that increased release of gastrointestinal hormones or factors which primarily stimulate enzyme synthesis and secretion may aggravate acute experimental pancreatitis while, on the other hand, release of hormones or factors primarily stimulating fluid secretion are without deleterious influence on the outcome of the disease. It is further obvious that drugs or agents having an inhibitory action on pancreatic secretion have not in controlled clinical studies been proved to have any beneficial effects in acute pancreatitis. This may indirectly reflect the findings of the present study that pancreatic secretion during the disease is already markedly depressed.

The role of hormonal stimulation in the pathogenesis of acute pancreatitis

In 1977, Lampel and Kern (81) reported that continuous intravenous infusion of caerulein caused an interstitial pancreatitis in the rat. It was characterized by a progressive, but reversible, interstitial oedema, marked elevation of S-amylase, decreased protein synthesis of the acinar cell and formation of large vacuoles in the cytoplasm which subsequently fused with the cell membrane and emptied by exocytosis to the interstitial spaces.

In the present series of investigations caerulein - but not secretin - was found to aggravate experimental pancreatitis. This aggravation was accompanied by changes in activities of digestive enzymes but also of lysosomal enzymes within pancreatic tissue and ascites (see page 28). As lysosomal enzymes, especially cathepsin, have been suggested to play a pathogenetic role in acute pancreatitis by contributing to the activation of trypsinogen within pancreatic tissue, special interest was focused on the influence of hormonal stimulation on lysosomal enzyme activities in normal and pancreatitic rats. Thus, the lysosomal enzymes cathepsin D, N-acetyl- β -D-glucosaminidase and acid phosphatase were studied concomitantly with the digestive enzymes amylase and lipase. In normal rats caerulein stimulation caused an increase within pancreatic tissue of the activities of cathepsin D (Fig 5) and N-acetyl- β -D-glucosaminidase, whereas the activity of acid phosphatase was unaffected. After caerulein administration amylase and lipase activities decreased. The protein concentration in pancreatic homogenates was uninfluenced. Further it was observed that caerulein induced an increased output of cathepsin D in bile-pancreatic juice. This finding suggests that cathepsin D may be associated with certain zymogen granules and secreted in a similar manner as digestive enzymes (amylase). Stimulation with secretin did neither affect the activities of lysosomal or digestive enzymes nor the protein concentrations in the pancreas.

During pancreatitis, the activities of cathepsin D (Fig 5), N-acetyl- β -D-glucosaminidase and amylase increased within pancreatic tissue whereas acid phosphatase decreased as compared to normal controls. Pancreatitic rats given caerulein displayed an increase of the activities within pancreatic tissue of cathepsin D (Fig 5) and also of acid phosphatase, whereas N-acetyl- β -D-glucosaminidase was unaffected and the amylase activity decreased. Also in ascites of pancreatitic rats caerulein administration caused a marked increase of the activities of cathepsin D (Fig 5) and amylase whereas the activities of the two other lysosomal enzymes studied were unaffected. The finding of increased cathepsin D values in caerulein-aggravated pancreatitis seem to support the hypothesis that this enzyme may be involved in the pathogenesis of acute pancreatitis.

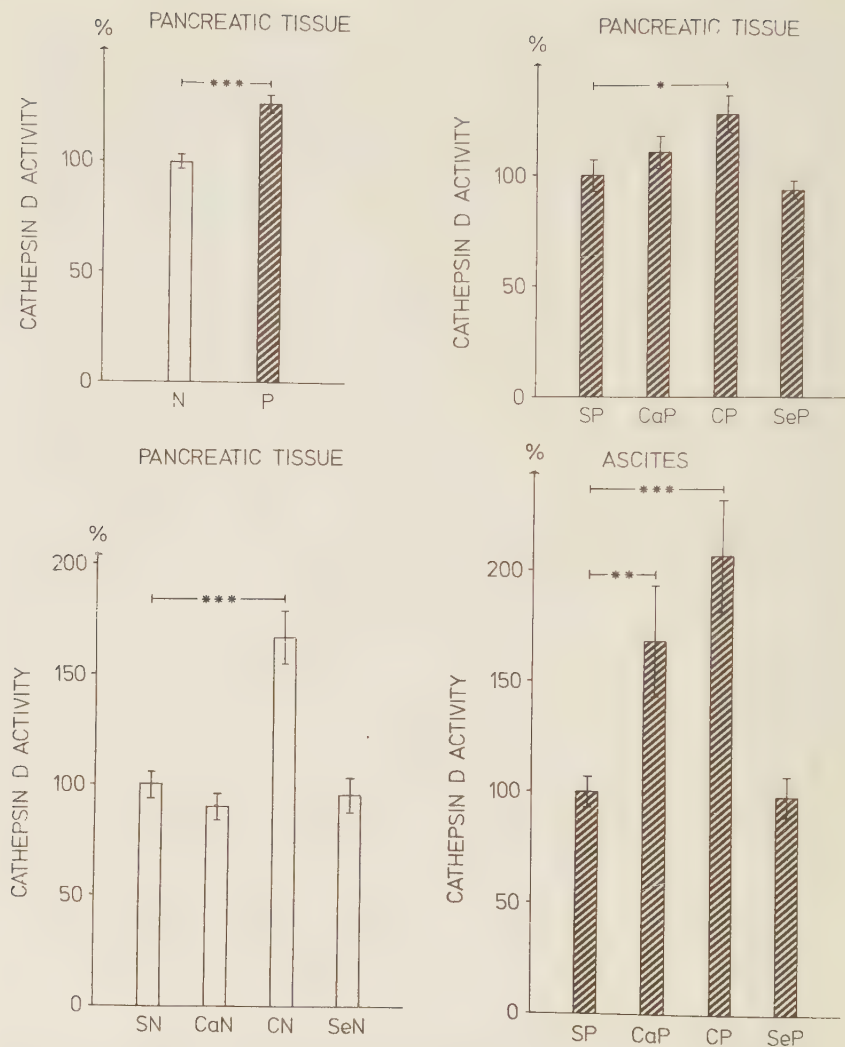


Fig 5. Activity of cathepsin D in pancreatic tissue of normal (N) and pancreatic (P) rats given repeated subcutaneous injections of saline (S), carbachol (Ca), caerulein (C) or secretin (Se) and activity of cathepsin D in ascites of pancreatic rats given saline (S), carbachol (Ca), caerulein (C) or secretin (Se). Activities are given as normalized values from the experiments described in paper VII and VIII. Mean $\pm$ SEM. Probability level of random difference is marked by x = $p < 0.05$, xx = $p < 0.01$ and xxx = $p < 0.001$.

In bile-induced pancreatitis in dogs, Dlugosz et al (33) observed an augmented lysosomal fragility in pancreatic tissue but did not find any apparent changes of the total activities of lysosomal hydrolases as compared to healthy controls. On the contrary, Rao et al (122) found increased total activities of cathepsin D, β -galactosidase, and acid phosphatase in pancreatic homogenates of mice with acute haemorrhagic pancreatic necrosis induced by feeding of choline-deficient ethionine-supplemented diet (0.5% DL). In mice Rao et al (122) found increased activities of amylase and trypsinogen, whereas Manabe and Steer (95) using a similar model found uninfluenced activities of these two enzymes in pancreatic tissue. Harrell et al (57) found in guinea-pig and Huttunen (61) in the rat decreased trypsinogen activities in the pancreas, whereas Beck et al (17) recorded normal trypsinogen activities in the dog after induction of pancreatitis. Thus, the results of enzyme estimations in pancreatic tissue in acute experimental pancreatitis are highly contradictory. This might possibly be explained by species differences and models for induction of pancreatitis used, but may also reflect the degree of pancreatitis and the time after induction at which the specimens or samples were taken. It also emphasizes a need for meticulous standardization of the model of induction as well as the method of evaluation when studying such a dynamic disease as acute pancreatitis.

The results of the present investigation show that in normal rats stimulation with caerulein causes changes of cathepsin D in pancreatic tissue similar to those obtained in acute experimental pancreatitis. Whether these observations are consistent with an action of lysosomal enzymes under the development of acute pancreatitis and/or whether the changes reflect a lysosomal role in the secretory process of the pancreatic acinar cell needs further investigations. However, a role for CCK over-stimulation in the pathogenesis of acute pancreatitis seems possible.

INFLUENCE OF CHOLINERGIC STIMULATION ON ACUTE PANCREATITIS^{*}

The normal exocrine pancreatic function is regulated by hormonal and neural stimuli, the complex interactions of which are still mainly unknown. Vagal (cholinergic) stimulation has been found to exert stimulatory effects both of secretion and synthesis of pancreatic enzymes (100, 161). Acetylcholine, which is released from the cholinergic nerve terminals following stimulation, acts on a cholinergic muscarinic receptor on the acinar cell. This receptor is different from that of cholecystokinin (161). However, cholinergic stimulation seems to lead to a similar change of calcium and cyclic 3,5-GMP as after CCK-stimulation resulting in synthesis and secretion of digestive enzymes (133).

Generally, the effect of vagal stimulation on the exocrine pancreas seems to be mediated in three different ways. Vagal stimulation of the stomach results in release of hydrochloric acid leading to a decrease of duodenal pH, which is known to stimulate the release of secretin from the upper intestinal mucosa. Further, ingestion of food leads to gastric distension evoking vago-vagal reflexes stimulating the release of antral gastrin, a hormone with stimulating effects on the acinar cell. Vagal stimulation may also act directly on the acinar cell. Atropine has the ability to block the acetylcholine receptors, thereby preventing these effects of vagal stimulation on the acinar cell but also the release of hydrochloric acid and gastrin from the gastric mucosa.

Effects of cholinergic stimulation on the course of acute pancreatitis

Inhibition of neural stimulation of the gland is regarded an important feature in the treatment of acute pancreatitis. Such treatment would be successful only on the assumption that vagal tonus is maintained or increased during the disease and that increased vagal stimulation is harmful. These matters have, however, not been sufficiently considered.

In the present series of experiments, a study was done on the influence of stimulation with the cholinergic agonist carbachol on the course of induced pancreatitis of mild severity. Rats were given carbachol subcutaneously immediately after induction of pancreatitis and after 3, 6 and 9 hours, whereas control pancreatic rats received saline at the same time intervals. The rats were observed for 25 hours and the mortality rate calculated for

^{*} The results presented refer to those of papers V, VII, VIII.

each group. Administration of carbachol resulted in significantly increased mortality rates as compared to control rats during the experimental period (Fig 3). The mechanism by which carbachol exerts its harmful effects is not known. One theoretical explanation may be an increased secretin stimulation of the exocrine pancreas as a consequence of lowered duodenal pH after carbachol stimulation of gastric hydrochloric secretion. This is, however, not probable as exogenous secretin stimulation in the present study was found not to be harmful in acute pancreatitis. Another hypothetical explanation is that carbachol augments the release of antral gastrin, which in turn stimulates the acinar cells leading to an increased mortality rate by mechanisms similar to those of caerulein stimulation. This explanation is, however, also unlikely as the physiological influence of gastrin stimulation on pancreatic function in healthy humans and rats has been questioned (100). It therefore seems most plausible that the increased mortality rate in pancreatitic rats following carbachol stimulation is caused by a direct cholinergic effect on the exocrine pancreatic cells.

As lysosomal enzymes may be involved in the pathogenesis and pathophysiology of acute pancreatitis the activities of lysosomal and digestive enzymes in pancreatic tissue and ascites were studied in pancreatitic rats given carbachol or saline as described above. This was done in an attempt to recognize any correlation between these enzyme activities and the severity of the disease. The activities of acid phosphatase in pancreatic homogenate increased whereas the activities of cathepsin D, N-acetyl- β -D-glucosaminidase and amylase were uninfluenced by carbachol stimulation (Fig 5). In ascites, the activities of amylase and cathepsin D increased after administration of carbachol, whereas acid phosphatase and N-acetyl- β -D-glucosaminidase were uninfluenced. As acute pancreatitis was aggravated by both caerulein and carbachol stimulation, and as the activity of cathepsin D in ascites was increased after both carbachol and caerulein stimulation, it seems reasonable to credit this enzyme a possible role in the pathogenesis and pathophysiology of the disease. Therefore, in the treatment of the disease, the role of protease inhibitors, such as e.g. pepstatin and leupeptin, inactivating not only trypsin but also cathepsins should be elucidated. Actually, beneficial effects of leupeptin were recently observed in rats with experimental pancreatitis (73).

As the findings in the present study support the view that increased vagal tonus is harmful for the outcome of pancreatitis, the influence of two different drugs - often used to decrease or inhibit the effects of neural stimulation of the gland in pancreatitis - was elucidated as to their effects on mortality rate and digestive and lysosomal enzyme activities in pancreatic tissue and ascites. Rats with induced acute pancreatitis of moderate severity were given the anticholinergic drug propantheline bromide (Pro-Banthine[®]) immediately after induction of the disease and after another 3, 6 and 9 hours, whereas control rats with pancreatitis

received subcutaneous saline injections at the same time intervals. The mortality rate during the experimental period (25 hours) was not influenced by the anticholinergic drug. It was also found that the activities of digestive and lysosomal enzymes, except for a slight increase of pancreatic acid phosphatase, was uninfluenced by anticholinergic administration. The failure of the drug to influence the course of the disease in a beneficial way can not be explained by insufficient vagal blockade as the dose given in ancillary experiments was shown to inhibit exocrine pancreatic secretion in healthy rats. One possible explanation of the lack of beneficial effects of anticholinergic treatment would be that vagal tonus is of minor importance in the disease. Most probably, however, the findings reflect that the concept of inhibition of pancreatic secretion in acute pancreatitis is questionable.

Cimetidine has recently been suggested in the treatment of acute pancreatitis, primarily due to its potent inhibition of gastric acid secretion, thereby inhibiting secretin stimulation of the pancreas (15, 32). Studies on exocrine pancreatic secretion after administration of cimetidine are, however, contradictory. Halliday and Both (53) studied in vitro pancreatic secretion in the rabbit and found cimetidine to be a potent inhibitor of pancreatic protein secretion. Thjodleifsson and Wormsley (151) contrary to Galmiche et al (44) and to Cavallini et al (25) found cimetidine to decrease pancreatic exocrine secretion in man. In unpublished experiments it was found that cimetidine in the doses used in the experiments described below did not affect pancreatic secretion.

In the present study cimetidine was given by subcutaneous injections every eight hours to rats with induced pancreatitis of moderate severity starting immediately after induction of pancreatitis. One group of rats were given a clinical dose and another group a 10-fold higher pharmacological dose whereas control rats with pancreatitis received saline injections. During the experimental period the clinical dose of cimetidine did not influence mortality rate, whereas the pharmacological dose led to a significantly increased mortality. These results are in agreement with those of Hadas et al (51) who later reported a significantly higher mortality rate in pancreatitic rats treated with intravenous cimetidine. In a small series of patients Regan et al (124) found an increased morbidity in patients with pancreatitis treated with glucagon in combination with cimetidine. Further, pancreatitis was observed in rats given ulcerogenic secretagogues in combination with cimetidine (72). It has even been suggested that cimetidine treatment for duodenal ulcer might induce acute pancreatitis (162). The lack of beneficial influence of cimetidine treatment may principally be explained in the same way as was the lack of beneficial effects of anticholinergic treatment, as inhibition of secretin release from the upper intestinal mucosa is probably of no importance for the outcome of the disease.

In summary, evidence is presented that vagal stimulation by the cholinergic agonist carbachol increases mortality rate in acute pancreatitis. The mechanism by which cholinergic stimulation is harmful might be related to altered acinar secretion and activities of catheptic enzymes. Drugs counteracting the direct or indirect effects of neural stimulation of the gland, such as e.g. anticholinergics and cimetidine, have no beneficial influence on the course of the disease when studied in the present experimental conditions. It seems that the concept according to which anticholinergic drugs and cimetidine have been advocated must be questioned, especially as overstimulation with secretin or oral administration of hydrochloric acid was found not to influence the outcome of acute experimental pancreatitis.

The role of cholinergic factors in the pathogenesis of acute pancreatitis

Prolonged nervous stimulation (12) as well as repetitive injections of acetylcholine (86) have been shown to induce morphological changes of the pancreatic acinar cell. Already in 1929 Villaret et al (154) proposed that acute pancreatitis could be induced by injection of a cholinergic agent. Recently it was shown that intravenous infusion to healthy rats of high doses of carbamylcholine induced signs of interstitial pancreatitis similar to those seen after caerulein stimulation (2). In the present study was shown that cholinergic stimulation increased the mortality rate in induced pancreatitis and further that the increased mortality rate was accompanied by increased activity of cathepsin D in peritoneal fluid (Fig 5). Because of the possible role of lysosomal enzymes in the pathogenesis of acute pancreatitis the influence of cholinergic stimulation on the activities of lysosomal and digestive enzymes in pancreatic homogenates of healthy rats was also studied. The dose of carbachol used was found to significantly increase bile-pancreatic output of amylase but no apparent effect on output of cathepsin D was noted. No macroscopic signs of pancreatitis were observed. It was found that the activity of pancreatic amylase increased and the activity of acid phosphatase decreased whereas lipase, cathepsin D and N-acetyl- β -D-glucosaminidase were uninfluenced. Thus, the changes of pancreatic enzyme activities in healthy rats following cholinergic stimulation are not corresponding to the changes caused by pancreatitis per se (see page 29 and Fig 5). In conclusion, these findings are not consistent with an important role for vagal (cholinergic) stimulation in the pathogenesis of acute pancreatitis.

Chapter 7

REST OF THE PANCREAS - RIGHT OR WRONG - IN THE MANAGEMENT OF ACUTE PANCREATITIS

A main principle in the conservative treatment of acute pancreatitis is "to put the gland at rest". This has generally been accomplished by measures aiming at inhibition of hormonal and neural stimulation of the gland, such as refraining from oral feeding, use of nasogastric suction and administration of different drugs inhibiting pancreatic secretion (32, 105, 141). The idea of "rest of the pancreas" is based on clinical observations and on theoretical considerations. Thus, it is generally stated that restriction of food intake and administration of nasogastric suction improve the clinical course of the disease. Secretion has been regarded as an expression of activity of the gland, and therefore it has been suggested that inhibition of secretion might be of value. Also, it has been assumed that hormonal or neural (over-) stimulation may aggravate the disease. There are, however, no unambiguous clinical or experimental studies supporting these theories. The present thesis constitutes a series of experiments analysing the conditions pro or contra the concept of "rest of the pancreas".

Except for the studies by Dreiling (37), who performed secretin and CCK stimulated pancreatic function tests in patients with acute pancreatitis without any harmful side effects, there seems to be no studies available on the role of hormonal or neural stimulation in the disease. In the present study experiments were performed in which rats with induced acute pancreatitis were given repeated injections of the gastrointestinal hormones secretin and caerulein and of the cholinergic agonist carbachol. Further, the role of endogenous hormonal release was studied in rats with pancreatitis. As was previously pointed out (cf Chapter 5 and 6) exogenous stimulation with the cholecystokinin analogue caerulein or with carbachol significantly increased the mortality rate as compared to saline-treated, pancreatic control rats, whereas exogenous stimulation with secretin was without effects in this respect (Fig 3). On the other hand there was no influence on survival rate by evoked endogenous hormonal release in pancreatic rats after oral administration of a light pellet meal, olive oil or hydrochloric acid. These latter findings probably reflect an insufficient stimulation of the release of gastrointestinal hormones, especially as oral administration of a trypsin inhibitor, known to evoke a marked release of intestinal factors some of which may have cholecystokinin-like effects from the intestinal mucosa, significantly increased mortality rate in rats with pancreatitis.

* The results presented refer to those of papers I-VIII.

The mechanism(s) by which stimulation of the inflamed gland worsen pancreatitis are not clear. In healthy rats it has been shown that stimulation by excessive doses of caerulein (81) or acetylcholine (2) can induce structural changes of the acinar cells similar to those seen in acute pancreatitis. In the present experiments the influence of hormonal and cholinergic stimulation on digestive and lysosomal enzyme activities in pancreatic homogenates and in peritoneal fluid was studied in healthy and pancreatitic rats. The results indicate that release of cathepsin D and amylase into ascites is enhanced in both caerulein and carbachol stimulated pancreatitic rats. In healthy rats, the activity of cathepsin D increased in pancreatic homogenate after caerulein stimulation but not after carbachol stimulation. This may reflect that the mechanisms by which caerulein and carbachol induce structural changes in healthy acinar cells are different. Whether the observed increase of the activities of amylase and cathepsin D in ascites is related to the increased mortality following caerulein and carbachol stimulation or merely reflects cellular dissolution can not be definitely settled. From the experiments presented, it can, however, be concluded that stimulation of the gland with hormones or factors promoting not only secretion but especially synthesis of enzymes is harmful and should be avoided or inhibited in the disease. Whether inhibition of pancreatic secretion gives pancreatic rest in the sense that the autodigestive process is retarded is, however, not studied. Hitherto anticholinergic drugs have been widely used in order to inhibit pancreatic secretion in acute pancreatitis (11). To study if anticholinergic administration to healthy rats influenced pancreatic protein concentration and enzyme activities an experiment was performed in which different doses of propantheline bromide (Pro-Banthine^R) were administered subcutaneously three times daily for three days. The anticholinergic dose generally recommended in the treatment of the disease caused a significant increase of the pancreatic protein concentration and content of amylase. These results indicate that anticholinergic treatment - by decreasing pancreatic secretion without influencing protein synthesis - might lead to accumulation of enzymes within the pancreas, which may have a negative influence on the outcome of the disease. In another series of experiments the influence of anticholinergic administration on the course of the disease was studied in rats with pancreatitis of moderate severity (cf Chapter 6). During the experimental period the mortality was uninfluenced by the anticholinergic treatment. Thus, it seems that anticholinergic treatment has neither beneficial nor harmful effects on acute pancreatitis. It seems as if the lack of clinical evidence for its value and the results of this thesis support the view that there is no place for anticholinergic drugs in the treatment of acute pancreatitis (11, 24).

The use of cimetidine was recently proposed as an alternative way to inhibit secretin stimulation and secretion of the gland in pancreatitis (15, 32). Also, it has been stressed that this drug

does not have any of the side effects of anticholinergic drugs such as tachycardia, intestinal atonia and urinary retention. Therefore, an experiment was done (cf Chapter 6) in which rats with acute pancreatitis of moderate severity were given subcutaneous injections of cimetidine every eight hours for 25 hours. Using a dose of cimetidine that significantly reduces gastric acid secretion and thereby the release of secretin no influence on mortality was encountered. When, however, a pharmacological dose of cimetidine was used a significantly increased mortality rate was observed during the experimental period. Thus, the results of the present study, like those of clinical studies (22, 101), do not support a general use of cimetidine in acute pancreatitis. In selected cases of severe pancreatitis cimetidine may be of value as a prophylaxis against stress ulcer.

Several hormones, e.g. glucagon, somatostatin, calcitonin and vasopressin, are known to inhibit pancreatic secretion in healthy individuals. Accordingly, these hormones have been proposed and attempted in the treatment of acute pancreatitis. There are hitherto no clinical or experimental studies supporting their use in the disease (cf Chapter 5).

The discouraging results of treatment with drugs inhibiting pancreatic secretion may be explained by their inability to inhibit the protein synthesis of the gland; as mentioned above the inflammatory process in acute pancreatitis is mainly proteolytic. Another reason for the therapeutic failure of antisecretory drugs may be the observation that the gland does not secrete during pancreatitis (Fig 3). Thus, the whole concept of inhibition of pancreatic secretion as a treatment in acute pancreatitis should be questioned.

The results of the secretory studies should be interpreted in view of the existence of a negative feed-back system regulating exocrine pancreatic secretion. In the rat (48, 65, 90), pig (66) and probably man (64) pancreatic enzyme secretion is controlled by a negative feed-back regulation exerted by intraluminal trypsin activity. Thus, e.g. infusion of trypsin into the duodenum of rats with pancreatic fistulas results in a rapid decrease in pancreatic secretion, whereas addition of trypsin inhibitor to the infusate causes a marked increase of the secretion. It has been suggested that this regulatory system is mediated by the release of gastrointestinal factors, especially CCK, from the small intestinal mucosa. The impaired secretion during acute pancreatitis may therefore initiate an increased release of G-I factors one of which may be cholecystokinin from the intestinal mucosa. The findings of the harmful effects of caerulein stimulation and of oral trypsin inhibitor administration in pancreatitis may suggest a vicious circle in pancreatitis initiated and sustained by the impaired pancreatic secretion (low intestinal trypsin activities). One theoretical way to counteract this would be intraduodenal infusion of trypsin

during the disease.

In an experimental series pancreatitis of moderate and severe degree was induced in rats. One group of rats was treated by oral administration of a buffer solution containing trypsin immediately after induction of pancreatitis and after another three and six hours, whereas control rats received the buffer solution at the same time intervals. A third group of rats received the trypsin inhibitor (Trasylol^R) in exactly the same way. The experimental periods comprised 25 and 72 hours, respectively. In none of the experiments trypsin influenced mortality rate whereas administration of trypsin inhibitor, significantly increased mortality rate after 25 as well as 72 hours. The results suggest that elimination or reduction of the already low intestinal tryptic activity in acute pancreatitis - by oral administration of trypsin inhibitor - have negative effects on the outcome of the disease. On the other hand oral administration of trypsin - unexpectedly - was without beneficial effects. The results suggest that even small amounts of trypsin within the intestine - as in acute pancreatitis - will exert the feed-back inhibition of pancreatic secretion and that this inhibition is not made stronger by additional trypsin. Another possible explanation of the lack of beneficial effects of trypsin administration is that the trypsin-solution did not enter the duodenal lumen. This possibility was, however, contradicted by the results of a separate but otherwise identical experiment in which methylen-blue added to the trypsin solution was detected in the small intestine already a few minutes after the administration.

The role of nasogastric suction and food restriction in the treatment of acute pancreatitis are still to be discussed. There are three controlled clinical studies on the value of nasogastric suction in acute pancreatitis and these were all conducted in patients with mild pancreatitis (41, 87, 148). In none of these there were any beneficial effects reported by this treatment. Concerning refraining from food administration there are to my knowledge no controlled studies available. In a retrospective study Ranson found that early refeeding to patients with severe pancreatitis increased the morbidity and mortality (118). In the present study the influence of administration of 0.6 g pellet diet by oral intubation every eight hours to rats with pancreatitis of moderate severity was studied. Control pancreatitic rats were sham intubated. There was no difference in mortality rate during the experimental period. In the same study another group of pancreatitic rats were given 2.0 g pellet diet at the same intervals. The mortality of this group was, however, significantly increased as compared to control rats. The increased mortality rate was judged to reflect increased hormonal and cholinergic stimulation of the gland. These results, like those of Ranson (118), seem to suggest that food restriction is of value at least in severe cases.

In summary, it should once again be mentioned that in the treatment of acute pancreatitis the concept of "rest of the gland" has been a matter of course and has therefore never been questioned nor tested in experimental or clinical studies. In the present study stimulation of the pancreas with hormones of the CCK group was found to aggravate the disease. Stimulation with secretin did not influence the outcome of the disease. Contrary to secretin, CCK is known to exert trophic effects - including stimulation of protein synthesis - on the pancreas. As the inflammatory process in acute pancreatitis is characterized by proteolysis it may be suggested that CCK exerts its negative effects by stimulating protein (enzyme) synthesis of remaining healthy acinar cells. This means that rest of the pancreas is of value only when effects of CCK are concerned. As CCK and acetylcholine both increase enzyme synthesis the negative effects of carbachol on survival in acute experimental pancreatitis found in the present thesis may be explained at least partly by the same mechanisms as the effect of CCK. Thus, in the future procedures aiming at rest of the pancreas should ideally inhibit the release specifically of CCK and CCK-like factors and of cholinergic stimuli. Inhibition of secretin release is of no use. Studies on the effect on pancreotone - a recently detected factor with antitrophic effects on exocrine pancreas - are of utmost importance (56). Also, the search for other substances with inhibitory effects on CCK release might pave the way to future therapeutic tools to be used in acute pancreatitis.

SUMMARY

In conclusion, the main findings of the present thesis may be summarized as follows:

1. Induction of acute experimental pancreatitis causes a marked reduction of pancreatic secretory volume and enzyme output.
2. Based on pancreatographic studies the impaired pancreatic secretion during acute pancreatitis is rather a result of ductal and parenchymal injury than of ductal obstruction.
3. Stimulation of the pancreas by hormones of the CCK-group is harmful in pancreatitis. This is probably a consequence of concomitant stimulation of pancreatic enzyme synthesis.
4. Cholinergic stimulation aggravates acute experimental pancreatitis possibly by stimulation of pancreatic enzyme synthesis.
5. Oral ingestion of trypsin inhibitor, causing a marked release of CCK or CCK-like factors from the intestinal mucosa, results in an increased mortality in acute experimental pancreatitis. On the other hand, oral trypsin administration in excess is without beneficial influence on the outcome of the disease.
6. Secretin stimulation does not influence the course or outcome of acute experimental pancreatitis.
7. A state of hyperinsulinism exists in the early stages of acute pancreatitis, probably as a consequence of insulin leakage from damaged insulin cells.
8. Acute pancreatitis is accompanied by increased S-amylase levels, the magnitude of which, however, is not correlated to the severity of the disease.
9. In "clinical" doses cimetidine administration does not affect the course of acute experimental pancreatitis. When given in a "pharmacological" dose (10-fold increase) cimetidine causes a significant increase in mortality.
10. Anticholinergic drugs given in doses comparable to those used in patients with acute pancreatitis cause an increase of pancreatic enzyme activities within the gland of normal rats. This probably reflects decreased secretion but undiminished enzyme synthesis.
11. In pancreatitic rats anticholinergic treatment is without influence on the course of the disease.

12. A non-parallel alteration of lysosomal enzyme activities occurs in pancreatic tissue in acute pancreatitis.
13. Lysosomal enzymes such as cathepsin D may be involved in the pathogenesis and outcome of acute pancreatitis.
14. The concept of "rest of the pancreas" not only involves inhibition of pancreatic secretion but also inhibition of pancreatic protein synthesis. Such "rest" is achieved by suppression of the release of both hormones of the CCK-group and of cholinergic stimuli.

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